Research Article

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The presence of sporogonic stages of *Tetracapsuloides bryosalmonae* in Icelandic salmonids detected using *in situ* hybridisation

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Abstract: Proliferative kidney disease (PKD) is a widespread temperature-dependent disease in salmonids caused by the myxozoan parasite, *Tetracapsuloides bryosalmonae* (Canning, Curry, Feist, Longshaw et Okamura, 1999) (*Tb*). *Tb* has a two-host life cycle, involving fish as an intermediate host and freshwater bryozoans as the definitive host. Although salmonids are acknowledged as hosts for the parasite, it is less clear which fish species are active hosts in the life cycle of *Tb*. Differences in infection dynamics have been observed between some fish species, which are thought to be related to the existence of two main *Tb*-strains, the American and European. Iceland, having three species of indigenous salmonids and positioned geographically between Europe and North America, is an ideal location to study the natural development of *Tb* in wild fish. The main aim of this study was to determine the genetic origin of *Tb* in Iceland and confirm whether mature spores are produced in Icelandic salmonids. In this study, Icelandic salmonids were infected with the European *Tb*-strain. *In situ* hybridisation revealed that intraluminal sporogonic stages, including mature spores, were commonly observed in all three salmonid species. The presence of intraluminal stages has previously been confirmed in brown trout *Salmo trutta* Linnaeus and Atlantic salmon *S. salar* Linnaeus in Europe, but they have only been observed in Arctic charr *Salvelinus alpinus* (Linnaeus) in North America, infected by the local strain. This is, therefore, the first time that sporogonic stages have been observed in Arctic charr in Europe, where fish are infected with the European *Tb*-strain. Our data strongly suggest that all the three salmonid species inhabiting Icelandic waters serve as active hosts in the life cycle of *Tb*. However, for full confirmation, transmission trials are needed.

Keywords: proliferative kidney disease, PKD, Arctic charr, brown trout, Atlantic salmon, sporogony, salmonid, intratubular

Proliferative kidney disease (PKD) is a widespread temperature-dependent disease of salmonids which has caused significant problems in farmed and wild salmonids in North-America and Europe (Clifton-Hadley et al. 1984, Hedrick et al. 1993, Wahli et al. 2002, 2007, Sterud et al. 2007). Evidence from central and northern areas of Europe, e.g., Scandinavia, Iceland, Switzerland, Austria (Sterud et al. 2007, Kristmundsson et al. 2010, Vasemägi et al. 2017), and recently in rivers in Montana, USA (Hutchins et al. 2021), suggest that PKD is an emerging disease and plays a significant role in severe declines observed in various populations of wild salmonids.

The causative agent of PKD, *Tetracapsuloides bryosalmonae* (Canning, Curry, Feist, Longshaw et Okamura, 1999) (*Tb*), is a myxozoan parasite, which requires both bryozoans and salmonids to complete its complex life cycle (Canning et al. 1996, Okamura 1996, Morris and Adams 2006). It is generally acknowledged that fish exposed

to *Tb* spores at water temperatures below 12–15 °C, get infected but do not develop the disease (Ferguson 1981, Clifton-Hadley et al. 1984, 1986, Foott and Hedrick 1987). However, at prolonged higher temperatures the parasite can cause clinical signs of PKD, which are primarily characterised by swollen and abnormally large kidneys due to an extreme inflammatory response of the host characterised by massive proliferation of cells in the haematopoietic tissue of the kidney (Canning et al. 2002, Ferguson 2006).

Research trials, made under controlled conditions, suggest that infectious spores of *Tb* are released from the bryozoan hosts into the surrounding freshwater when the water temperatures exceed 7–8 °C (Gay et al. 2001). Subsequently, salmonids are exposed to the spherical and short-lived spores of the parasite (de Kinkelin et al. 2002, McGurk et al. 2005). The route of infections is generally thought to occur via the gills (Morris et al. 2000a, Grabner and El-Matbouli 2010), although penetration through the

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skin may also occur (Longshaw et al. 2002). When inside the fish, the spores migrate to their target organs, primarily the kidney, via the vascular system (Morris et al. 2000a,b, Longshaw et al. 2002, Grabner and El-Matbouli 2010). The initial development of the parasite in the fish occurs in the kidney interstitium (termed extrasporogonic stages). At a certain point during development, the parasite enters the kidney tubules, by penetrating the tubular epithelium, and initiates the sporogonic phase. An intratubular pseudoplasmodium is formed and subsequently develops into infective spores, which exit the fish host with urine (Kent and Hedrick 1985, Morris and Adams 2008).

Due to strong immune response of the fish infected with *Tb*, and in many cases the apparent absence of spore maturation, the possibility of some salmonid species being accidental hosts has long been suggested (Hedrick et al. 2004). Although spore-like stages in renal tubules and the urinary bladder have been observed in a number of salmonid species, the definitive identification of these form as *Tb* is commonly lacking (Kent and Hedrick 1986, Bucke et al. 1991, Feist and Bucke 1993, Kent et al. 2000). However, Hedrick et al. (2004), confirmed *Tb* spores from urine of rainbow trout in North America, with *in situ* hybridisation and IFAT using both monoclonal and polyclonal antibodies. Furthermore, a number of trials have failed to demonstrate transmission from fish to bryozoans, suggesting that some salmonids might be accidental or dead-end-host for the parasite and hence are not involved in maintaining its life cycle (Tops et al. 2004, Tops and Okamura 2005, Grabner and El-Matbouli 2008, Kumar et al. 2013).

The existence of two main strains of *Tb*, European and North American, is generally acknowledged (Henderson and Okamura 2004). These strains have adapted to infect different salmonid hosts; the European strain seems to be more adapted to species of the genus *Salmo* Linnaeus whilst the North American one to species of the genus *Oncorhynchus* Suckley (Bucke et al. 1991, Morris et al. 1997, Morris and Adams 2006, Kumar et al. 2013). Differences between strains of *Tb* with regard to parasite development in certain fish hosts have been observed, where some fish species appear to be suitable hosts for one strain of the parasite but not the other (Bucke et al. 1991, Kent et al. 2000, Grabner and El-Matbouli 2008, Morris and Adams 2008, Kumar et al. 2013). Therefore, the complete life cycle of *Tb*, regarding parasite strain, salmonid species and/or populations, remains unresolved in many aspects.

Considering the existence of two main *Tb* strains and the apparent difference in host-parasite relationship between different salmonids from Europe and North America, the geographic location of Iceland, as an isolated island in the North Atlantic between Europe and North America, makes Icelandic salmonids infected with *Tb* an interesting study object.

The aim of this study was to determine the genetic origin of *Tb* in Iceland using rDNA analysis and examine whether *Tb* spores are produced in the three native salmonid species in Iceland, i.e., Arctic charr *Salvelinus alpinus* (Linnaeus), brown trout *Salmo trutta* Linnaeus and Atlantic salmon *Salmo salar* Linnaeus.

MATERIALS AND METHODS

Sampling

In September 2009, Arctic charr and brown trout were caught in Lake Elliðavatn and Atlantic salmon in the River Elliðaár, the lake's outflowing river, located within or close to Reykjavík, the capital of Iceland. The Arctic charr and brown trout were caught using gill nets whereas Atlantic salmon were caught by electrofishing. The fish were subsequently brought to the laboratory, where they were euthanised with an overdose of MS-222. Following that, the fork length of the fish was determined. The fish were then dissected, samples from the posterior and anterior kidney were taken from 20 fish of each species and fixed in 10% buffered formalin and otoliths for age determination. The formalin-fixed samples were prepared for histological examination according to conventional protocols, i.e., embedded in paraffin wax, sectioned (3 µm) and stained with haematoxylin and eosin (HE). The HE-stained slides from all three fish hosts were examined under a compound microscope for the presence of *Tb*. Eight slides from each fish species, showing extrasporogonic and suspected intratubular stages of *Tb*, were selected for examination by *in situ* hybridisation (ISH). All Arctic charr and brown trout samples selected were from fish showing gross clinical signs of PKD and severe histopathological changes characterised by significant renal hyperplasia. All samples from Atlantic salmon came from fish with subclinical infections. In addition, kidney samples from Arctic charr and brown trout were fixed in 96% ethanol for molecular analyses.

Fish size and age

The fork length and age of the eight fish of each species, selected for further examination, were as follows. Atlantic salmon: mean length 11.9 cm (range 8.1–16.0 cm); mean age 1.4 years (range 0–1+ to 2+ year). Arctic charr: mean length 20.1 cm (15.0–28.1 cm); mean age 1.5 years (range 1–3+ years). Brown trout: mean length 18.4 cm (range 13.6–28.5 cm); mean age 1.6 years (range 1–3+ years).

In situ hybridisation

The ISH methodology (ISH) primarily followed the procedure of Morris et al. (1999), with some modifications. In brief, histological sections, 7 µm thick, were hydrated and permeabilised with 10 µm/ml proteinase K in Tris-buffered saline (TBS) pH 8 for 12 minutes at 37 °C followed by a 2 × 5 min washing in PBS. Samples were then post-fixed in 0.4% paraformaldehyde in PBS for 15 min and subsequently washed 2 × 5 min in distilled water. To prevent non-specific binding, sections were exposed to 10% hydrogen peroxide (H₂O₂) in methanol (CH₃OH) for 10 min and then washed 2 × 5 min in distilled water. Samples were then enclosed with Frame-Seal™ (Bio-Rad, Sundbyberg, Sweden) incubation chambers and equilibrated in a 'ready to use' hybridisation buffer (Roche, Mannheim, Germany, REF. 11717472001). Based on Morris et al. (2000a), who tested published *Tb* primers (Saulnier and de Kinkelin 1997, Kent et al. 1998) for their suitability for *in situ* hybridisation, the following three probes were used, all of which targeting the 18S rRNA gene: PKX1458 5' TAT CGG ATT ACT TCG TAC GC 3', PKX4R 5' CCG TTA CAA CCT TGT TAG GAA 3' and PKX6R 5' GGA CCT TAC TCG TTT CCG ACC 3' (Kent et al. 1998). The sections were sealed and

Fig. 1. Histological section through a kidney of Arctic charr severely infected with *Tetracapsuloides bryosalmonae* (Canning, Curry, Feist, Longshaw et Okamura, 1999) (*Tb*). **A** – *In situ* hybridisation (ISH) section showing extrasporegonic stages in the kidney interstitium (black arrow), pseudoplasmodia penetrating the tubular epithelium (white arrows) and intraluminal stages of *Tb* (arrowhead); **B** – higher magnification of pseudoplasmodia between epithelial cells (black arrow = nucleus of epithelial cell) of a kidney tubule (white arrow); **C**, **D** – higher magnification of intraluminal sporogonic stages of *Tb* (arrowhead) and extrasporegonic forms (black arrow); **E** – mature *Tb* spores in the lumen of a kidney tubule. Note the polar capsules (thin arrow) and the polar filaments inside them. **A**, **B**, **D**, **E** – *in situ* hybridisation sections; **C** – stained with haematoxylin-eosin.

denatured at 95 °C for 4 min followed by an 18 hour hybridisation at 45 °C. Hybridisation was followed by non-stringent and stringent washes with 2 × SSC and SSC with 0.1% Tween 20 °C at 42 to 45 °C, respectively. Signal detection was achieved using incubation with horseradish peroxidase-labelled streptavidin (Dako, Agilent Technologies, Glostrup, Denmark) for 20 min at room temperature followed by 3 × 5 min washing in PBS (pH 7.4) and visualised with a DAB Peroxidase Substrate (Vector Laboratories, Burlingame, USA). Haematoxylin was applied

as a counterstain, after which sections were rapidly dehydrated in a series of ethanol, transferred to xylol and mounted in resin-based medium.

DNA analyses

Total DNA from 20 mg of each kidney sample was extracted from three different fish for both Arctic charr and brown trout, using GeneMatrix Tissue DNA Purification Kit (EURx, Gdansk, Poland) following the tissue protocol. Ribosomal DNA (rDNA),

Fig. 2. Histological section through a kidney of brown trout and Atlantic salmon infected with *Tetracapsuloides bryosalmonae* (Canning, Curry, Feist, Longshaw et Okamura, 1999) (*Tb*). Mature intraluminal spores of *Tb* (arrowheads) in brown trout (**A**, **B**) and Atlantic salmon (**C**, **D**). Inserts – higher magnification of mature *Tb* spores in brown trout (**B**) and Atlantic salmon (**D**). Each spore has two spherical polar capsules (thin arrows). The polar filament inside the capsule can be seen in the spore in the Atlantic salmon (white arrowhead). **A**, **C** – stained with haematoxylin-eosin; **B**, **D** – *in situ* hybridisation sections.

including partial 18S, full ITS1 and partial 5.8S of *Tb* was amplified using the primers PKD-1700f 5'-AGC GAG AAC TTG GTG GTA GC-3' and PKD-5.8mhr 5'-CGC AGC AAG CTG CGT TCT TCA TCG A-3' designed from alignments of myxozoans made in CLUSTAL-X (Thompson et al. 1997). PCR conditions were as follows: denaturing step of 95 °C for 5 minutes followed by 34 cycles of 94 °C for 30 seconds, 60 °C annealing for 30 seconds and another 30 seconds at 72 °C. This was completed with 72 °C for 7 minutes for final extension. DNA sequencing was performed in both forward and reverse directions for all PCR products and nucleotide BLAST searches in the GenBank database, performed for each sequence read to confirm a malacosporean origin. The contiguous sequences were obtained manually using CLUSTAL X and BioEdit.

RESULTS

In situ hybridisation (ISH)

Successful ISH was achieved on kidney samples from all three salmonid species examined. A high abundance of extrasporogonic stages of *Tb* was observed in the kidney interstitium of both Arctic charr and brown trout (Fig. 1A). In addition, pre-sporogonic *Tb* forms were commonly seen between epithelial cells, especially in Arctic charr, in

which they penetrated during their migration into the tubular lumen of the kidney (Fig. 1B). Sporogonic stages were seen inside kidney tubules of all three salmonid species, often in high numbers and at different stages of development, including what appeared to be mature spores with polar capsules (Figs. 1C–E, 2A–D). While extrasporogonic stages in the haematopoietic tissue were few in Atlantic salmon, even absent in some fish, intraluminal sporogonic stages of *Tb* were very common.

DNA analysis

Identical ribosomal DNA sequences were successfully obtained from both brown trout (Genbank accession number: MN831894) and Arctic charr (MN831895). They were 100% similar to *Tb* strains (AJ639976 and AJ639990) isolated from *Salmo trutta* in the United Kingdom (Hampshire – England, UK) and *Fredericella sultana* (Blumenbach) (Dorset – England, UK), respectively.

DISCUSSION

Intraluminal sporogonic stages, including mature spores, were detected by ISH in all three salmonid species in Iceland. This strongly suggests that all the three native salmonids inhabiting Icelandic freshwater are active hosts for this

malacosporean parasite. Furthermore, results from molecular analyses confirm that salmonids in Lake Elliðavatn in SW Iceland are infected with the European strain of *Tb*, as the rDNA was identical to that of strains isolated from the UK.

It is commonly acknowledged that salmonids are susceptible to infections with *Tb*, together with at least one non-salmonid species, the northern pike, *Esox lucius* Linnaeus. However, what is less clear is which of these fish species are active hosts in the life cycle of *Tb*. Numerous researchers have speculated over the years, on both sides of the North Atlantic, whether some, or even all, salmonids could be aberrant/dead-end hosts for this parasite (Seagrave et al. 1980a,b, Kent and Hedrick 1985, 1986). Initially, the argument for these speculations was the lack of intraluminal sporogonic stages of the parasite (Hedrick et al. 1993). Later, after the discovery of the definitive bryozoan host (Canning et al. 1996, 2002), unsuccessful transmission trials suggested that some salmonid species were not active hosts while others confirmed the role of some salmonids as true hosts (e.g., Tops et al. 2004, Tops and Okamura 2005, Grabner and El-Matbouli 2008, Kumar et al. 2013). What complicated things further is the existence of two strains of *Tb*, the European and North American ones, the latter one considered to be more diverse and primordial (Henderson and Okamura 2004). These strains appear to have local adaptation to certain salmonids. The European strain seems to be adapted to species of the genus *Salmo* and the North American strain to species of the genus *Oncorhynchus* (see Bucke et al. 1991, Morris et al. 1997, Morris and Adams 2006, Kumar et al. 2013). The genus *Salmo* includes fish species, which are mainly native to Europe whereas the genus *Oncorhynchus* is mostly comprised of native salmon and trout species occurring in North America. Although rainbow trout *Oncorhynchus mykiss* (Walbaum) is common in Europe (mostly farmed) and anadromous *Salmo trutta* (sea trout) is present in North America, these are non-native introduced species in both continents. In contrast, both Arctic charr and Atlantic salmon are native to both European and North American waters (Froese and Pauly 2020).

To date, successful transmission of *Tb* from infected fish to naïve bryozoans has been demonstrated in brown trout and brook trout, *Salvelinus fontinalis* (Mitchill), in Europe (Morris and Adams 2006, 2008, Grabner and El-Matbouli 2008, Kumar et al. 2013, Abd-Elfattah et al. 2014). Furthermore, intraluminal sporogonic stages of the parasite have been detected in numerous fish species, suggesting that they are active hosts in the life cycle of *Tb*. These include brown trout, Atlantic salmon, Arctic charr, brook trout, grayling (*Thymallus thymallus* (Linnaeus)), northern pike and at least eight species of *Oncorhynchus* in North America, i.e., species of Pacific salmon, rainbow trout and cutthroat trout (Arkush and Hedrick 1990, Braden et al. 2010, Gorgoglione et al. 2020). In some of these studies, the authors stated that only immature forms were detected and not mature spores, whereas mature spores with visible polar capsules were observed in others (Bucke et al. 1991, Feist and Bucke 1993, Morris et al. 2000b, Hedrick et al. 2004, Grabner and El-Matbouli 2008).

The absence of mature spores allowed speculation on whether it could be due to the inability of the parasite to produce mature spores in these hosts, which then could be considered dead-end hosts (Hedrick et al. 1993). Therefore, successful fish to bryozoan transmission trials must be performed to be able to make a definitive statement that a certain fish species is without doubt an active host. However, transmission trials have been performed with relatively few fish species. Most of these were on brown trout and rainbow trout (Morris and Adams 2006, 2008, Grabner and El-Matbouli 2008, Abd-Elfattah et al. 2014, Kumar et al. 2013), but also on brook trout, grayling and northern pike, in a single study (Grabner and El-Matbouli 2008). Apparently, no such trials have been conducted on Arctic charr, Atlantic salmon or any fish inhabiting North America.

The presence of sporogonic stages in brown trout and Atlantic salmon in Icelandic salmonids, presented in this study, agrees with previous reports. As noted earlier, brown trout is one of the two species, which are fully confirmed active hosts in the life cycle of *Tb* in Europe, with intraluminal sporogonic stages commonly observed and a number of successful fish-to-bryozoan transmission trials. Similarly, although not supported by transmission trials, intraluminal sporogonic stages have been reported from Atlantic salmon in several studies, using conventional histology and immunohistochemistry, suggesting it is also an active host (Bucke et al. 1991, Morris et al. 2000b, Mo et al. 2011). In contrast to the above, the presence of mature *Tb* spores in Arctic char is more unexpected, as it is the first report of intraluminal forms in this salmonid species of the European strain of *Tb*.

Differences between strains of *Tb* with regard to fish hosts have been encountered, where some species appear to be active hosts for one strain of the parasite but not for the other (Bucke et al. 1991, Kent et al. 2000, Grabner and El-Matbouli 2008, Morris and Adams 2008, Kumar et al. 2013). The two known examples involve Arctic charr and rainbow trout inhabiting the two continents. Numerous researchers suggest that rainbow trout is not an active host in the life cycle of the European strain. Intraluminal sporogonic forms have never been detected and all transmission trials have been unsuccessful (Grabner and El-Matbouli 2008, Kumar et al. 2013). In contrast, the presence of intraluminal forms observed in rainbow trout in North America suggests that this fish species is an active host in the life cycle of the American strain. That furthermore indicates that the American strain is adapted to rainbow trout, whereas the European one is not. These findings could be considered expected as this fish species is not native to European waters and therefore no long-term host-parasite co-evolution exists.

The Arctic charr example is a bit more unclear. Intraluminal forms, including mature spores, have been detected in Arctic charr in North America (British Columbia) (Kent et al. 2000), but not in Europe, until now. Unlike rainbow trout, Arctic charr has a circumpolar distribution and is therefore native to both European and North American waters. Considering available data, two explanations

seem plausible, genetic differences between Arctic charr populations with different host-parasite relationship and limited research on Arctic charr, both in Europe and North America. Phylogenetic data on Arctic charr indicate that extensive genetic differences exist among northern European populations of Arctic charr, both on a larger scale, between geographically distinct areas, but also on a smaller scale, e.g., between lakes in a distinct isolated island like Iceland and even within the same lake (Wilson et al. 2004). Research performed on two different strains of Arctic charr in Scotland (Anonymous 2001a) showed a striking disparity in terms of *Tb* infections. The two Arctic charr strains, which were kept in adjacent tanks, were both naturally infected with enzootic water. Whilst one of the fish strains was highly susceptible to infections, acquiring heavy *Tb* infections and suffering high mortality (98%), the other strain did not develop any PKD and examination of kidney samples from these fish by PCR did not show any evidence of the parasite. The authors concluded that this might suggest strain variation among Arctic charr to developing PKD.

It is fair to assume that similar differences could exist in terms of spore formation. Therefore, the absence of intratubular forms of *Tb* in Arctic charr in Europe might be affected by the very limited *Tb* research on Arctic charr. Possibly, sporogonic forms would be detected if a number of different Arctic charr strains would be examined. That, however, does not change the fact that our results are in contrast with available data from Arctic charr in the UK, in which sporogonic stages have not been observed (Morris and Adams 2008), or in other European countries, for that matter, suggesting that this salmonid species might be a dead-end-host for the European strain of *Tb*. Furthermore, this fish species has been considered highly susceptible to the disease (Brown et al. 1991, Anonymous 2001a) which has led to considerations whether that might be an indication of a short host-parasite coevolution and the possibility of this species being a facultative or accidental host.

The species-poor Icelandic fish fauna can further support the role of Icelandic Arctic charr in the life cycle of the parasite. Due to Iceland's geographic location, as an isolated island in the middle of the North Atlantic Ocean, its post-glacial recolonisation of freshwater fish has been limited to anadromous (salmonids and stickleback) and catadromous (eel) species. Consequently, only five native freshwater fish species exist in Iceland, either as land-locked in lakes or migrating to sea, i.e., Arctic charr, brown trout, Atlantic salmon, European eel (*Anguilla anguilla* (Linnaeus)) and three-spined stickleback (*Gasterosteus aculeatus* Linnaeus) (Anonymous 2001b). When the land rose again, due to melting of the ice, many of these anadromous fish became landlocked, i.e., lost the possibility of sea migration. Consequently, numerous lakes in Iceland are at present only inhabited by one salmonid species, in

particular, land-locked Arctic charr. This greatly simplifies things in terms of possible fish hosts for *Tb*. In terms of the definitive host of *Tb*, five bryozoan species have been found in Icelandic freshwater. Species of the genera *Fredericella* Gervais and *Plumatella* Lamarck, which are well known hosts for *Tb* (Anderson et al. 1999, Longshaw et al. 1999, Okamura and Woods 2002), are the most common species and are found in almost every lake examined (Kristmundsson and Magnúsdóttir 2015). In terms of *Tb*, bryozoans in Icelandic waters are poorly studied. *Tb* spores have, however, been found in *Fredericella sultana* in Lake Elliðavatn (unpublished data), i.e., the lake under study here. Observations on the presence of *Tb* in two such lakes in Iceland showed that 40–80% of Arctic charr were infected with *Tb* (Svavarsdóttir 2016). The bryozoan species *Plumatella repens* Linnaeus has been reported from one of those lakes (Kristmundsson and Magnúsdóttir 2015). However, although it has been shown that infected bryozoans have the ability to pass covert *Tb* infections to their distribution forms, the statoblasts (Hartikainen et al. 2013, Abd-Elfattah et al. 2014), it seems highly unlikely that merely bryozoans could sustain such a degree of *Tb* in these lakes, in the absence of an active fish host.

Although much has been revealed in the life cycle of *Tb*, particularly after the bryozoan definitive hosts were discovered in the late 1990s, much remains unclear. The *Tb* hosts, but also different strains of the parasite itself, form parts of this puzzle. Therefore, more thorough understanding of the infection dynamics between the different fish hosts and different strains of the parasite itself, is still lacking. The limited freshwater fish fauna in Iceland limits presumable fish species as active hosts for *Tb*. In addition to three-spined stickleback and European eel, the Icelandic freshwater fish fauna merely consists of three salmonid species, i.e., Atlantic salmon, Arctic charr and brown/sea trout (Anonymous 2001b). No research exists, indicating that three-spined sticklebacks and eels are hosts for *Tb*, neither active nor dead-end ones. Brown trout is a fully confirmed host for *Tb* and all research suggest that Atlantic salmon is also an active host (e.g., Bucke et al. 1991, Morris et al. 2000b, Morris and Adams 2008). The findings of the present study strongly suggest that all salmonids native to Icelandic freshwater fauna are active hosts in the life cycle of the European strain of *Tb*. However, for full confirmation, transmission studies are needed.

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